



## GST-Theta1 Enzyme Expression Levels in Brain Tumor and Their Relationship with the Clinical Data of the Patients

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### Abstract

The most important drug metabolizing enzyme of the detoxification mechanism is known as Glutathione S-Transferase. GST enzymes may be associated with brain tumor epidemiology, clinical and demographic factors. The correlation between parameters such as changes in Glutathione S-transferase Theta1 proteins, tumor localizations, age, gender, alcohol use, smoking, chemotherapeutic/radiotherapeutic treatment status in normal and brain tumor tissues, diagnosed in neurosurgery department, were examined by immunohistochemistry. GST-Theta1 expressions were analyzed using immunostaining in samples from 149 patients diagnosed with brain tumors between 2016 and 2018. The mean age of the patient group was 49.44 years. 83 (58.45%) patients were male. After immunohistochemical staining, GST-T1 expression was found approximately 9,46 times higher in tumor tissues than in normal tissues ( $p < 0.0001$ ). Tumor tissues from patients who received chemotherapy showed higher expression of GST-T1 than those who did not ( $p < 0,05$ ). In addition, GST-T1 expression level was observed at a significantly higher level in patients younger than 60 years of age compared to patients over 60 years ( $p < 0.026$ ;  $p < 0.05$ ). There was no statistically significant relationship between patients smoking behaviors,

alcohol consumption, tumor localization and GST-T1 expressions. It is aimed to determine the GST-T1 protein expressions and to contribute to the examination of epidemiological and prognostic factors of brain tumors by comparing them with demographic and clinical data.

**Key words:** Brain cancer, Immunohistochemistry, Glutathione-S Transferase, GST-T1

## 1. Introduction

Recently, human, who is trying to adapt to chemical exposure, increasing stress and technological lifestyle, can provide some tolerance against all this exposure due to her metabolic structure (Courtens et al., 1996; Rustoen et al., 1999). It is known that such increased exposures cause the formation of some metabolic and autoimmune diseases when the dose exceeds the tolerance threshold in metabolism. Cancer is one of these diseases (Watson and Killgore, 2016; Jörg et al., 2016). Its incidence and prevalence are increasing rapidly in our country and all over the World (Ferlay et al., 2019). Although it is in the last place in terms of incidence among cancer types, the epidemiology of brain tumor, which has a critical importance in terms of treatment and surgical intervention, is the subject of some studies today as a very important issue (Darlix et al., 2017; Barnholtz et al., 2018; Ostrom et al., 2019). This organ, which is in the first place in terms of importance in the central nervous system, is also very important in terms of being the place where all metabolic activities are controlled and the signals received from the signal transmission system are collected (Ostrom et al., 2017). Any damage to this important organ is likely to result in loss of function, partial or permanent paralysis, or death (Nabetani and Shintaku, 2018; Castello et al., 2018; Okidi et al., 2020; Sarasso et al., 2021). Therefore, current research has gained a lot of importance epidemiologically in order to improve the existing treatment methods in cancer disease or to find new treatment approaches that do not have permanent effects and have a high success rate (Mendes et al., 2018; Wu et al., 2018; Park et al., 2021). On the other hand, the most important known factor of cancer formation mechanisms is exposure to toxic substances (Wang et al., 2018; Kumar et al., 2019). It is known that chemical compounds and elements that cause cytotoxic effects and oxidative stress cause the degradation of the molecular structure and uncontrolled proliferation in the irreversible cell cycle (Ji et al., 2021). One of the tolerance mechanisms in metabolism is the xenobiotic mechanism, which proceeds with the principle of chemical reduction and removal of these toxic agents to their water-soluble state. This mechanism is also known as the detoxification mechanism (Adwas et al., 2019; Hayat et al., 2021). Glutathione S Transferases (GSTs), located in Phase II, where the most important reactions in this mechanism take place, are located in different subcellular compartments, including the cytosol, mitochondria, endoplasmic reticulum, nucleus, and plasma membrane. GSTs have important effects on cell growth, oxidative stress as well as disease progression (Raza, 2011). Oxidative stress plays a role in the pathogenesis of multifactorial diseases. GSTs are involved in the cellular detoxification process and neutralizing oxidative stress. Glutathione S-transferase Theta1 (GST-T1) is involved in cellular detoxification and is thought to have a determining effect on the susceptibility to tumor formation and disease severity (Townsend et al., 2005; Askari et al., 2018; Singh and Reindl, 2021). It can be thought that whether it is related to the expression levels of GST-T1 isoenzyme in the tissues of patients with Brain Tumors, it

is important for a better understanding of the development of the disease. In our study, the results of GST-T1 expression, which we obtained by applying immunohistochemical method in brain tumor and normal tissues of the patients, were evaluated comparatively with some clinical and demographic data, and we aimed to reveal the relationships between the results.

## **2. Material and Methods**

Clinical information about patients operated for intracranial tumors was reported by the members of the Gulhane Training and Research Hospital Neurosurgery Clinic. The archived clinical data of these patients, diagnosed between 2017 and 2019, were retrospectively evaluated. All data contains information about patient demography, histopathology and protein enzyme expressions. The written permissions were obtained from the ethics committee of the Health Sciences University Keçiören Education and Research Hospital for the entire study. All resections were performed by neurosurgeons according to a standard procedure, and clinical data were obtained by a neurosurgeon. GST-T1 were analyzed in tumor tissues of patients immunohistochemically. In addition, these markers were analyzed in normal brain tissue samples obtained from the surrounding tissue. Expression levels of the subjected proteins were classified as 0, 1, 2 or 3 based on microscopic examination after immune staining.

### **2.1. Histopathological examination**

A standard protocol was followed for pathological examination of resected samples. The archived, diagnosed samples of neurosurgery department and other clinical data were examined macroscopically by a pathologist in each case. From some patients, peripheral healthy tissue surrounding the tumor were also resected to be used as negative control. Only intracranial tumors (including gliomas, metastases, meningiomas, and pituitary adenomas) were included in the data set. Where more than one tumor was found, all were removed and examined. Tumor size was measured as the largest surface size. Blocks were taken to show maximum direct tumor growth of the brain. Additional blocks were specifically taken to demonstrate the relationship between the tumor and any constructive structure or tissue, and the lines of resection and normal brain tissue. Tumor grade, differentiation and degree of anaplasia were evaluated considering the nature of tumor margin (push or infiltration) and the presence and importance of brain invasion. All the pathological features analyzed were investigated in each sample and their presence or absence was recorded in detail. In the study, paired distant neighbor samples of the tumor tissue were used with a portion of the normal brain tissue surrounding the tumor site to observe the GST-T1 expression status in normal brain tissue regions to form a control group. Clinical data of patients; gender, age, smoking, alcohol use, tumor localization, chemotherapy status, radiotherapy status and ranking according to the data of the World Health Organization were included in the study as clinical parameters.

### **2.2. Immunohistochemical (IHC) staining**

Tissues were fixed in 10% buffered formalin and embedded in paraffin blocks. 4  $\mu$ m thick sections were cut and a section was picked to be stained with hematoxylin and eosin (HE) to observe tissue morphology. For

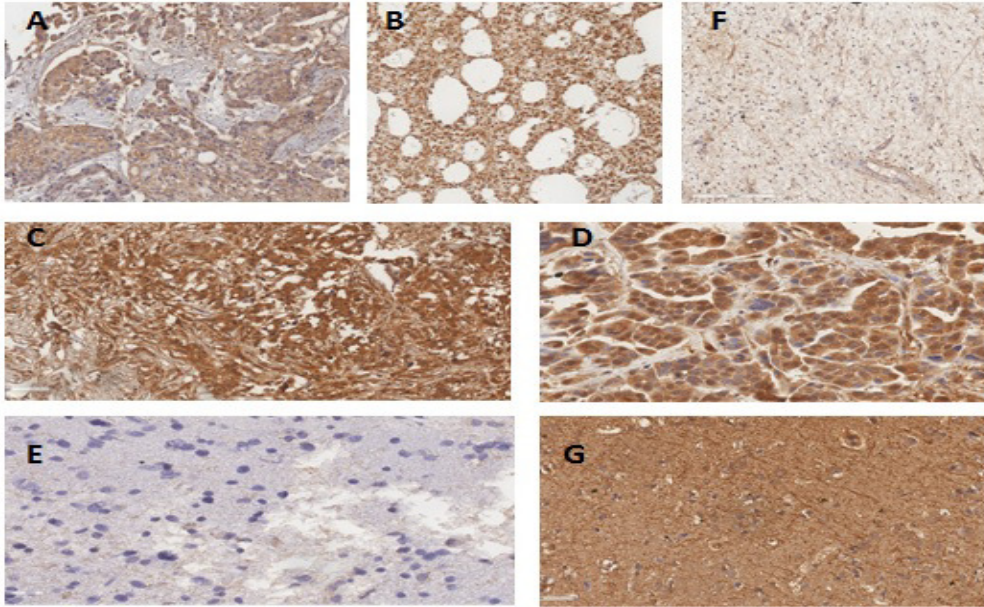
immunohistochemistry, endogenous peroxidase activity was blocked by incubating sections in 1% hydrogen peroxide (v / v) in methanol for 10 minutes at room temperature. The sections were then washed in distilled water for 5 minutes and antigen was taken in a household pressure cooker for 3 minutes using 0.01 M citrate buffer (pH 6.0). After washing with distilled water, the sections were transferred into 0.05 M Tris-HCl (pH 7.6) containing 0.15 M sodium chloride (TBS). Sections were incubated with Super Block (streptavidin / HRP complex [SHP125]; ScyTek Laboratories USA) to prevent nonspecific background staining at room temperature for 10 minutes. Sections were then coated with primary antibodies diluted 1:50 for anti-GSTT1, at 4 °C, a hour (anti GST-Theta1 from Cloud Clone Corp. TX, USA). After washing for 15 minutes in TBS, the sections were incubated for biotinylated link antibody followed by SHP125 at room temperature. Diaminobenzidine was used to visualize peroxidase activity in tissues. Nuclei were lightly counterstained with hematoxylin, and then the sections were dehydrated and mounted. Both positive and negative controls were included in each study. Light microscopy of immunohistochemically stained sections was performed by patients' clinical information, other histopathological data or pathologist unaware of survival. Tissue nuclei from the central part of the tumor and from the invasive front were evaluated separately in each sample, such as the presence of nuclear and cytoplasmic staining in tumor epithelial cells. The staining intensity was graded as 0 (no staining), 1 (poor staining), 2 (medium staining) or 3 (strong staining).

### **2.3. Statistical analysis**

All statistical analysis was done using SPSS v25.0. Continuous data were expressed as mean±standart error of mean (SEM) and categorical was number (n) and percentage (%). Staining scores were calculated according to the intensity of positively stained tumor tissues. Staining intensity was graded as; 0: negative expression, +1 weak expression, +2: moderate expression, +3: strong expression. The normality of data was assessed using the Shapiro Wilk and Kolmogorov-Smirnov tests. Levene variance test was also performed. Mann Whitney-U was used to examine the differences between two independent groups as a non-parametric test. Independent Samples-Kruskal Wallis-H test following Bonferroni correction was used to assess the differences between more than two independent groups with non-normal distributions. Spearman's rank correlation coefficients were computed for correlation analyses. A p-value of less than 0,05 was accepted as statistically significant.

### **3. Results**

The preparative samples obtained as a result of the GST-T1 marker application with the immunohistochemical method were examined by a specialist pathologist by microscopy and expression scoring was performed. General images of some samples obtained microscopically are as in Figure 1.



**Figure 1.** (A) Shows GST-T1 (+1) positive nuclei which are stained brown in tumor tissue (x4). (B) Shows GST-T1 (+2) positive nuclei which are stained brown in tumor tissue (x8). (C) Shows GST-T1 (+3) positive nuclei which are stained brown in tumor tissue (x10). (D) Shows GST-T1 (+3) positive nuclei which are stained brown in tumor tissue (x40). (E) Shows GST-T1 (-) negative nuclei which are not stained with a colour in normal tissue (x40). (F) Shows GST-T1 (+1) positive nuclei which are stained brown in normal tissue (x20). (G) Shows GST-T1 (+2) positive nuclei which are stained brown in normal tissue (x40).

Demographic data such as gender, age, smoking status, alcohol consumption are shown in Table 1. 149 brain tumor and normal tissue specimens were analyzed in this study. The average age of the patients was 49.44 at a range of 6 to 83 years. Female patients comprised 41.7% of the samples, while 58.3% were male. A total of 30.2% of patients were smokers, and 10.1% of drank alcohol.

**Table 1.** Patients' demographic data.

Demographic data	n (%)
<b>Gender</b>	
Female	62 (41.7%)
Male	87 (58.3%)
<b>Age (years)</b>	
<60	96 (64.43%)
≥60	53 (35.57%)
<b>Smoking</b>	
Yes	45 (30.2%)
No	103 (69.1%)
Missing	1 (0.7%)
<b>Alcohol consumption</b>	
Yes	15 (10%)
No	133 (89.3%)
Missing	1 (0.7%)

n: Number, %100 percentage of total population

Clinical data including the characteristics of the tumors, radiotherapy and chemotherapy status, information about surgical procedures such as number, part, resection margin, lesion localization are exhibited in Table 2. According to clinical data, 55 patients (36.9%) received radiotherapy and 32 (21.5%) were chemotherapy. 113 patients (75.8%) were operated on once. Most tissue was collected from the frontal lobe with 28.9% following by parietal lob with 13.4%.

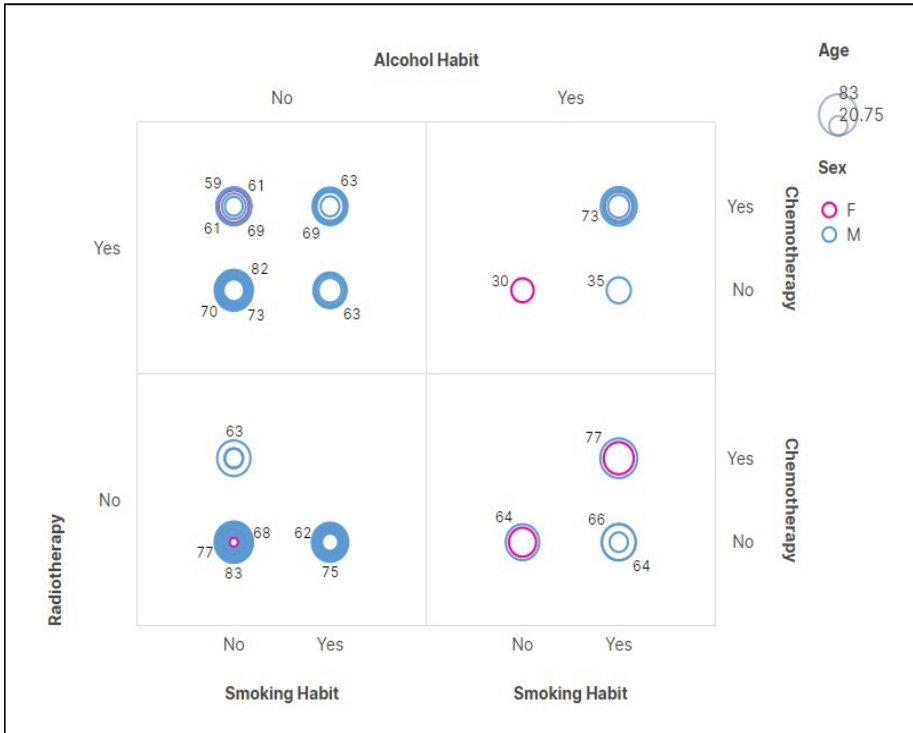
**Table 2.** Patients' clinical data.

<b>Clinical data</b>	<b>n (%)</b>
<b>Chemotherapy</b>	
Yes	32 (21.4%)
No	116 (77.9%)
Missing	1 (0.7 %)
<b>Radiotherapy</b>	
Yes	55 (36.9%)
No	92 (61.7%)
Missing	2 (1.4%)
<b>Number of surgery</b>	
1.00	113 (75.8%)
2.00	30 (20.1%)
3.00	2 (1.3 %)
4.00	1 (0.7%)
5.00	1 (0.7%)
7.00	1 (0.7%)
10.00	1 (0.7%)
<b>Part</b>	
Bilateral	1 (0.7%)
Middle	24 (16.1%)
Left	66 (44.3%)
Right	58 (38.9%)
<b>Resection margin</b>	
Gross total	91 (61.1%)
Parsiel	5 (3.4%)
Subtotal	52 (34.8%)
Missing	1 (0.7%)
<b>Lesion localization</b>	
Frontal	48 (32.6%)
Parietal	26 (17.3%)
Temporal	22 (14.7%)
Cerebellar	18 (12%)
Occipital	10 (6.7%)
Other	25 (16.7%)
<b>Pathology</b>	
Glial tumor	46 (30.8%)
Metastasis	42 (28.2%)

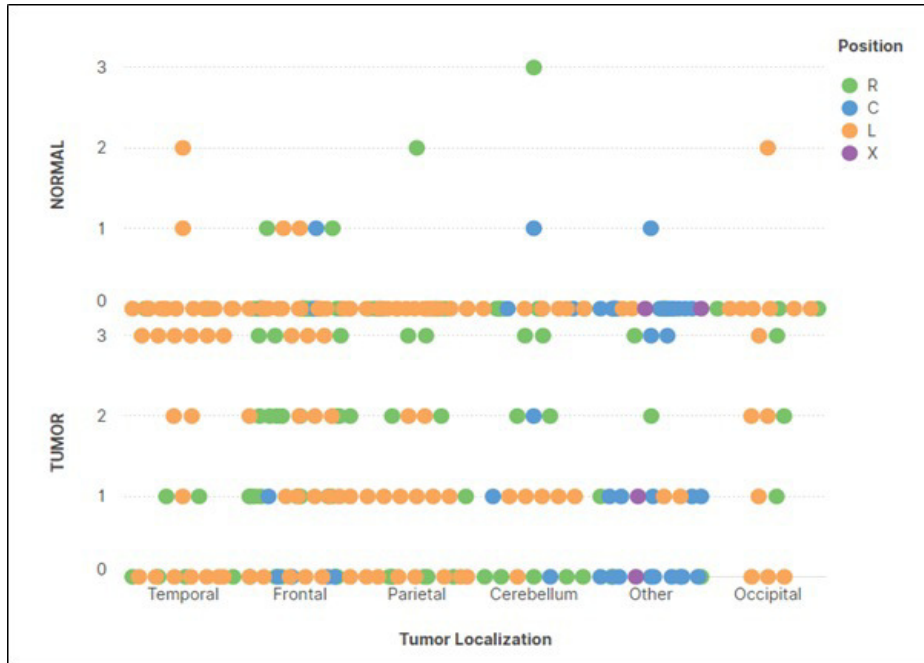
Meningioma	32 (21.6%)
Pituitary Adenoma	12 (8.0%)
Radiation Necrosis	3 (2.0%)
Schwannoma	3 (2.0%)
Cavernoma	2 (1.3 %)
Medulloblastoma	2 (1.3 %)
Central neurocytoma	2 (1.3 %)
Demyelinating disease	1 (0.7 %)
DNET	1 (0.7 %)
Inflammation	1 (0.7 %)
Craniopharyngioma	1 (0.7 %)
Lhermitte Duclos Disease	1 (0.7 %)

n: Number, 100% percentage of total population

Demographic data and general representation of clinical data of 149 patients with brain tumors included in our study are shown in Figure 2 and Figure 3.



**Figure 2.** Demographic information distribution of patients according to Gender, age, alcohol and cigarette use, radiotherapy and chemotherapy treatment status.



**Figure 3.** Distribution of the rates of GST-T1 enzyme expression in normal and tumor tissues, depending on the main localizations of the brain.

GST-T1 expression profiling of brain tumor and normal tissues are presented in Table 3. 28.19% of tumor tissues exhibited weak expression, while 5.37% of normal tissues were. 16.78% of tumor tissues and 2.01% of normal tissues showed moderate expression. 14.09% of tumor tissues and 0.67% of normal tissues showed strong expression. Tumor tissues presented almost 9.46 fold higher GST-T1 expression than normal tissues. Tumor tissues showed significantly greater expression than normal tissues ( $p < 0.0001$ ).

**Table 3.** GST-T1 expression profiling of brain tumor and normal tissues.

Staining Scores	GST-T1	
	Tumor	Normal
0	61 (40.94%)	137 (91.95%)
1	42 (28.19%)	8 (5.37%)
2	25 (16.78%)	3 (2.01%)
3	21 (14.09%)	1 (0.67 %)
<b>Total</b>	149 (100%)	149 (100%)
<b>Mean</b>	1.04 ± 0.09 <sup>a</sup> (0-3) <sup>b</sup>	0.11 ± 0.04 <sup>a</sup> (0-3) <sup>b</sup>
<b>T/N p-value</b>	<b>9.46</b> <b>&lt;0.0001*</b>	

Staining scores were calculated according to the intensity of positively stained tissues. Staining intensity was graded as; 0: negative expression, +1: weak expression, +2: moderate expression, +3: strong expression a: mean ± sem; b: minimum and maximum staining intensity p values less than 0,05 were considered statistically significant



GST-T1 expressions in brain tumor and normal tissues according to patients' demographic characteristics were exhibited in Table 4. These demographic characteristics are age, sex, smoking, and alcohol consumption status. GST-T1 expression in tumor tissues taken from patients younger than 60 years of age was found to be higher than those of 60 years and older, significantly ( $p < 0.026$ ;  $p < 0.05$ ). No significant differences were observed in GST-T1 expression in regards to gender, as well as smoking and alcohol consumption habits ( $p > 0.05$ ).

**Table 4.** GST-T1 expressions in brain tumor and normal tissues according to patients' demographic characteristics.

Variable	GST-T1	
	Tumor	Normal
<b>Age</b>		
<60	1.19 ± 0.11 <sup>a</sup> (0-3) <sup>b</sup>	0.16 ± 0.05 <sup>a</sup> (0-3) <sup>b</sup>
≥60	0.77 ± 0.13 <sup>a</sup> (0-3) <sup>b</sup>	0.04 ± 0.03 <sup>a</sup> (0-1) <sup>b</sup>
p-value	0.026	0.146
<b>Gender</b>		
Female	1.02 ± 0.15 <sup>a</sup> (0-3) <sup>b</sup>	0.08 ± 0.06 <sup>a</sup> (0-3) <sup>b</sup>
Male	1.06 ± 0.11 <sup>a</sup> (0-3) <sup>b</sup>	0.13 ± 0.04 <sup>a</sup> (0-2) <sup>b</sup>
p-value	0.638	0.107
<b>Smoking</b>		
Yes	1.04 ± 0.16 <sup>a</sup> (0-3) <sup>b</sup>	0.13 ± 0.06 <sup>a</sup> (0-2) <sup>b</sup>
No	1.05 ± 0.11 <sup>a</sup> (0-3) <sup>b</sup>	0.11 ± 0.04 <sup>a</sup> (0-3) <sup>b</sup>
p-value	0.989	0.400
<b>Alcohol consumption</b>		
Yes	0.93 ± 0.28 <sup>a</sup> (0-3) <sup>b</sup>	-
No	1.06 ± 0.09 <sup>a</sup> (0-3) <sup>b</sup>	0.13 ± 0.04 <sup>a</sup> (0-3) <sup>b</sup>
p-value	0.630	0.227

Staining scores were calculated according to the intensity of positively stained tissues. Staining intensity was graded as; 0: negative expression, +1 weak expression, +2: moderate expression, +3: strong expression a: mean ± sem; b: minimum and maximum staining intensity. p values less than 0,05 were considered statistically significant.

GST-T1 expressions in brain tumor and normal tissues depending on patients' clinical characteristics are shown in Table 5. These demographic characteristics are radiotherapy, chemotherapy, lesion localization, and post-op status. Tumor tissues taken from the patients treated with chemotherapy possessed significantly higher GST-T1 expression than those who did not receive chemotherapy ( $p < 0.05$ ). There are no statistically significant differences between groups depending on radiotherapy and lesion localization ( $p > 0.05$ ). GST-T1 expressions of tumor tissues taken from post-op exitus patients were statistically greater than that of alive patients ( $p = 0.004$ ;  $p < 0.05$ ).

**Table 5.** GST-T1 expressions in brain tumor and normal tissues according to patients' clinical characteristics.

Variable	GST-T1	
	Tumor	Normal
<b>Chemotherapy</b>		
Yes	1.36 ± 0.19 <sup>a</sup> (0-3) <sup>b</sup>	0.06 ± 0.06 <sup>a</sup> (0-2) <sup>b</sup>
No	0.95 ± 0.10 <sup>a</sup> (0-3) <sup>b</sup>	0.13 ± 0.04 <sup>a</sup> (0-3) <sup>b</sup>
p-value	<b>0.041</b>	0.245
<b>Radiotherapy</b>		
Yes	1.11 ± 0.13 <sup>a</sup> (0-3) <sup>b</sup>	0.14 ± 0.07 <sup>a</sup> (0-3) <sup>b</sup>
No	1.00 ± 0.12 <sup>a</sup> (0-3) <sup>b</sup>	0.10 ± 0.04 <sup>a</sup> (0-2) <sup>b</sup>
p-value	0.358	0.781
<b>Lesion localization</b>		
Frontal	1.30 ± 0.15 <sup>a</sup> (0-3) <sup>b</sup>	0.09 ± 0.04 <sup>a</sup> (0-1) <sup>b</sup>
Parietal	0.75 ± 0.75 <sup>a</sup> (0-3) <sup>b</sup>	-
Cerebellar	1.00 ± 0.28 <sup>a</sup> (0-3) <sup>b</sup>	0.27 ± 0.21 <sup>a</sup> (0-3) <sup>b</sup>
Temporal	1.06 ± 0.31 <sup>a</sup> (0-3) <sup>b</sup>	0.12 ± 0.12 <sup>a</sup> (0-2) <sup>b</sup>
Other	0.90 ± 0.12 <sup>a</sup> (0-3) <sup>b</sup>	0.10 ± 0.05 <sup>a</sup> (0-2) <sup>b</sup>
p-value	0.254	0.880
<b>Post-op status</b>		
Alive	0.86 ± 0.10 <sup>a</sup> (0-3) <sup>b</sup>	0.13 ± 0.05 <sup>a</sup> (0-3) <sup>b</sup>
Ex	1.38 ± 0.16 <sup>a</sup> (0-3) <sup>b</sup>	0.08 ± 0.05 <sup>a</sup> (0-2) <sup>b</sup>
p-value	<b>0.004</b>	0.452

Staining scores were calculated according to the intensity of positively stained tissues. Staining intensity was graded as; 0: negative expression, +1 weak expression, +2: moderate expression, +3: strong expression a: mean ± sem; b: minimum and maximum staining intensity. p values less than 0,05 were considered statistically significant.

Correlation analysis was performed between GST-T1 expression levels and the other continuous variables. No significant correlation was found between the variables (p>0.05).

#### 4. Discussion

In order to understand the metabolism of xenobiotics in the detoxification mechanism, the effects of these enzymes on drug resistance, free oxygen radicals, oxidative stress and carcinogenesis have been the subject of research in the literature.

Murray and Burke (1995) reported that the best method for determining drug metabolizing and detoxifying enzymes in the cell content is the immunohistochemical method.

In a 2003 study to determine the expression of p125 in paraffin blocks of tumor tissues, 18 different colorectal liver metastases as well as 24 pairs of primary colorectal carcinoma and colorectal liver metastases were examined using immunohistochemical tests using monoclonal anti-FAK 4.47. They stated that there was high p125 FAK expression in normal colorectal mucosa and both primary colorectal tumors and liver metastatic tumors (Lark et al., 2003).

In a study on GST-P isoenzyme expression and carcinogenicity in 1993, GST-P expression level in liver cells of 194 pesticides (Diethylnitrosamine, S-tributylphosphorothioate, Vinclozolin, Chlorobenzilate, Tecnazene, Isoproturon, Dicloran) sample applied at various concentrations was controlled by immunohistochemical method. There was no difference in the expression of GST-P after administration, which could lead to a tendency to carcinogenicity. It was also reported that no significant difference was observed between the chemicals applied (Hoshiya et al., 1993).

In another study, in which the expression levels of GST isozymes alpha, mu and pi were examined by immunohistochemical method in 51 patients with head and neck tumors treated with chemotherapy, it was stated that the cytotoxic effects of chemotherapy may cause irreversible damage. It was observed that the blind scores obtained with the GST-P antibody gave the same result as the pan-specific antibody in all cases except one (Nishimura et al., 1996).

In a study conducted in 2018 with 55 patients diagnosed with intracranial brain tumor by immunohistochemical method, cytochrome p450 (CYO) and GST-P1 enzyme expression levels were examined. A significant increase in enzyme expression was detected. In addition, the highest GST and CYP enzyme levels were observed in pituitary adenomas. Therefore, they reported that these tumor types are able to metabolize more drugs than other intracranial tumors (Kural et al., 2018).

In 2013, a study with mouse asthma models emphasized the importance of GSTs in the detoxification of ROS compounds and toxic compounds and examined their correlation with asthma. They showed increased expression of GST especially in the group treated with OVA and PBS. It was noted that mRNA expression for GST-P1 was higher than for PBS in patients treated with OVA. It has been reported that there is no significant difference between GST-P1, GST-T1, GST-M1 in terms of expression levels. It has also been noted that increased oxidative stress causes upregulation of GSTs (Sohn et al., 2013).

In the case diagnosed with breast cancer in 2003, 67 (58%) samples were GST-P and 43 (37%) cases were Bcl2 positive. When examined by double immunofluorescence labeling, significant accumulation of GST-P marker was observed in the nuclei of cancer cells (Huang et al., 2003).

GST Omega expression levels were investigated in various human tissues such as glial, breast myoepithelial, colon neuroendocrine cells, fetal myocytes, hepatocytes, biliary epithelial cells, pancreatic ductal epithelium, Hoffbauer cells in the placenta, C cells in the thyroid tissue, and follicular cells. It has been reported that the condensation in the nucleus depending on the expression in the cells is similar to the known expression states of other GST isozymes, as well as in GST Omega. It has been suggested that the functions of GST Omega that differ from other isozymes should be revealed (Yin et al., 2003).

In another study examining the resistance of glutathione group compounds to chemotherapy-active agents used in brain tumors in the literature, they reported that the resistance was more pronounced in irregular isozyme and evenly distributed metabolism (Backos et al., 2012).

Expression levels of GST isozymes in kidney cells were investigated by immunohistochemistry method in 1989. Heterogeneous and multifocal were observed in all 12 kidney tissues using GST-alpha, GST-pi and GST-mu. It has also been stated that this situation may be associated with carcinogenesis (Harrison et al., 1989).

The chemoresistance of GST-pi, GST-mu and GST-alpha isozymes was followed in a study by grafting A549 non-small cell lung cell lines on mouse models. As a result of the findings of the study, it was stated that the A549 xenograft model could be used to determine which of the drugs used in hospitals plays a more active role (Mathieu et al., 2004).

In another study comparing CYP1A1 and GSTP1 enzyme expression levels in brain tumor tissues, Western blot and Elisa techniques were used in a study conducted with 36 patients with brain tumors and a control group in the Pakistani population. CYP1A1 expression was observed 4 times higher in tumor tissues than in normal tissues. GST-P expression level was found to be lower in tumor tissues than in normal tissues (Wahid et al., 2013).

GST-P expression levels after exposure to Cytokeratin, which is the subject of another study in the literature, were examined by SELDI-TOF mass spectrophotometer and confirmed by immunohistochemical method and qPCR. CK8 phosphorylation at Ser73 and Ser431 has been reported to be strongly associated with hepatocellular carcinoma and carcinogenesis. Strong expression was also expressed at the mRNA level in validation studies performed in qPCR. GST-P has been shown to be strongly induced in proliferation and tumor-focused regions (Kakehashi et al., 2009).

GST-alpha, GST-mu and GST-pi by immunohistochemical method at different stages of human ovarian cells (different stages of menstrual cycle and pregnancy and after menopause) due to intracellular transport of steroids and their drug metabolizing roles enzyme expression levels were examined. Different expression levels were observed in different stages of the ovarian cell. However, it has been observed to be significantly associated with the GST-dependent 5,3-ketosteroidisomerase, which catalyzes the conversion of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione (Rahilly et al., 1991).

The expression levels of glutathione-related compounds of platinum-based chemotherapy treatments used in head and neck tumors were the subject of another study. Immunohistochemistry and in situ hybridization techniques were used in the study conducted in 51 patients. It has been observed that patients with a low GST score are 4.7 times more likely to respond to chemotherapy treatment than patients with a high GST score (Nishimura et al., 1998).

A study in 1997, the expression level of GST-P enzyme in benign and malignant prostate tissues was examined by immunohistochemical method. In the study conducted with 69 benign and 44 malignant prostate tissues, it was detected in basal cells in benign tissues at a rate of 67%. It has been reported that weak positive expression was observed in 94% of other epithelial cells (Cookson et al., 1997).

GST-P enzyme activity in endocrine cells in colorectal cancer tissues was investigated. It has been determined that cases with GST-P-expressing endocrine cells in primary tumor tissues have a worse prognosis after surgical intervention than those with GST-P negative endocrine cells (Gulubova and Vlaykova, 2010).

Expression levels of GST-P enzyme in human glioma cells were compared with Pgp, MRP1, MRP3, MRP5 enzymes. MRP1, MRP5 and GST-P enzyme expressions were predominantly observed in endothelial cells and drug resistance was reported to be high in these cases with high enzyme activity (Calatuzzolo et al., 2010).

In 2008, GST-alpha, GST-pi and GST-mu enzyme expression levels in the brain tissues of epileptic patients were investigated. GST-alpha expression was not observed in the study conducted with 32 epileptic patients and 8 normal control groups. Expression of GST-mu was observed in 63% of the control group and 53% of the epileptic samples. It was determined that GST-pi expression was observed at a rate of 50% in the control group in endothelial and glial cells, while it was synthesized at a rate of 66% in epileptic cells (Shang et al., 2008).

GST-P enzyme expressions in 150 human glioma cells were investigated by immunohistochemical method. While a gradual increase in GST-P expression was observed, no significant association was found with age, gender, or other clinical data (Wang et al., 2017).

The data obtained in our study were made by applying the method of immunohistochemistry, which is the preferred method in other studies in the literature, on brain tumor tissues. From these data, the GST-T1 expression level observed in tumor tissues was found to be quite high compared to normal tissues. In addition, significantly higher expression levels were found in individuals over 60 years of age and in patients receiving chemotherapy treatment.

## **5. Conclusion**

The most important known role of phase II enzymes, which play a role in detoxification and drug metabolism mechanism, is to detoxify chemotherapeutic agents used in cancer treatment. In this direction, the fact that the data in this section obtained in our study yield similar results causes us to evaluate this role of GST enzymes with its known contribution. We think that the increase in GST-T1 expression in metabolic processes related to carcinogenesis in elderly individuals will be a factor in the diagnosis and epidemiological understanding of brain tumors. In addition, we suggest the importance of the necessity of clarifying whether these enzyme expressions are affected by damages at the genetic level or whether it is genetically controlled by other studies to be carried out.

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## Conflicts of Interests

Authors declare that there is no conflict of interests

## Ethics committee permission

Ethics committee approval for this scientific research study was obtained from the Health Sciences University Ethics Committee with the decision numbered 2012-KAEK-15/1810.

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